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Nandy et al.

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(54) **DNA SEQUENCE, AND RECOMBINANT PREPARATION OF GROUP 4 MAJOR ALLERGENS FROM CEREALS**

(75) Inventors: **Andreas Nandy**, Hamburg (DE); **Helmut Fiebig**, Schwarzenbek (DE); **Oliver Cromwell**, Suesel-Fassendorf (DE)

(73) Assignee: **MERCK PATENT GMBH**, Darmstadt (DE)

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C12N 1/20	(2006.01)
C12N 15/00	(2006.01)
C12N 15/09	(2006.01)
C12P 21/04	(2006.01)
C12P 21/06	(2006.01)
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(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,762,943 A	6/1998	Dolovich et al.
7,214,786 B2 *	5/2007	Kovalic et al. 536/23.6
2005/0074464 A1	4/2005	Deweerd
2006/0177470 A1	8/2006	Fiebig et al.

FOREIGN PATENT DOCUMENTS

WO	WO 03025008	3/2003
WO	WO 2004/000881	12/2003
WO	WO 04000881	12/2003

OTHER PUBLICATIONS

Genbank Accession No. AP003544.1 published in 2001.*
Meinkoth et al. 'Hybridization of nucleic acids immobilized on solid supports.' Anal. Biochem. 138:267-284, 1984.*
Tarzi et al., "Peptide Immunotherapy for Allergic Disease", Expert Opinion Biol. Therap., 3 (4): 617-626, 2003.
Gavrovic et al., "Microheterogeneity Examination of Grass Group 4 Allergens", Allergy, 53 (Suppl. 43): 27, 1998.
Stumvoll S et al: "Purification, Structural and Immunological Characterization of a Timothy Grass (*Phleum pratense*) Pollen Allergen, Phl P 4, With Cross-Reactive Potential" Biological Chemistry, Bd. 383, Nr. 9, Sep. 2002, XP002260346.
Astwood JD et al: "Cloning and Expression Pattern of Hor V 9, The Group 9 Pollen Isoallergen From Barley" Gene: An International Journal of Genes and Genomes, Elsevier Science Publishers, Barking, GB, Bd. 182, Nr. 1-2 Dec. 5 1996, XP004071930.
Rihs H P et al: "Polymerase Chain Reaction Based cDNA Cloning of Wheat Profilin: A Potential Plant Allergen" International Archives of Allergy and Immunology, Bd. 105, Jan. 1994, XP000604627.
Fahlbusch B et al: Detection and Quantification of Group 4 Allergens in Grass Pollen Extracts Using Monoclonal Antibodies: Clinical and Experimental Allergy, Blackwell Scientific Publications, London, GB, Bd. 28, Nr. 7, Jul. 1998, XP002260345.
Database EMBL'Online! 19. Nov. 2004 XP002325590.
Database EMBL 19. Nov. 2004, XP002325591.
Database ENBL 19. Nov. 2004, XP002332685.
Database ENBL 19. Nov. 2004, XP002332686.
Database EMBL 19. Nov. 2004, XP002332687.
Martis et al. "The Genome Structure of Rye (*Secale cereale* L.) and Its Multiple Parents." W400. Plant & Animal Genome XXI; San Diego, CA. Jan. 14, 2013.
Li et al. "High levels of nucleotide diversity and fast decline of linkage disequilibrium in rye (*Secale cereale* L.) genes involved in frost response." BMC Plant Biology, 11:6, 2011.
Becker et al. "Dna sequence and recombinant production of the grass pollen allergen phl p4". Publication No. WO2004000881 A1—Publication Date: Dec. 31, 2003—(English Machine Translation).

* cited by examiner

Primary Examiner — Nora Rooney

(74) *Attorney, Agent, or Firm* — Millen, White, Zelano, Branigan, P.C.

(57) **ABSTRACT**

The present invention relates to the provision of DNA sequences of group 4 major allergens from cereals. The invention also encompasses fragments, new combinations of partial sequences and point mutants having a hypoallergenic action. The recombinant DNA molecules and the derived polypeptides, fragments, new combinations of partial sequences and variants can be utilized for the therapy of pollen-allergic diseases. The proteins prepared by recombinant methods can be employed for in vitro and in vivo diagnosis of pollen allergies.

11 Claims, No Drawings

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**DNA SEQUENCE, AND RECOMBINANT
PREPARATION OF GROUP 4 MAJOR
ALLERGENS FROM CEREALS**

This application is a divisional of U.S. patent application Ser. No. 10/583,089, filed on Jun. 15, 2006, which issued as U.S. Pat. No. 7,935,347 on May 3, 2011, which is a US National Phase under §371 of PCT/EP04/13664, filed on Dec. 1, 2004, the disclosures in which are incorporated by reference herein in their entirety.

BACKGROUND OF THE INVENTION

The present invention relates to the provision of DNA sequences of group 4 major allergens from cereals (Triticeae). The invention also encompasses fragments, new combinations of partial sequences and point mutants having a hypoallergenic action. The recombinant DNA molecules and the derived polypeptides, fragments, new combinations of partial sequences and variants can be utilised for the therapy of pollen-allergic diseases. The proteins prepared by recombinant methods can be employed for in vitro and in vivo diagnosis of pollen allergies.

Type 1 allergies are of importance worldwide. Up to 20% of the population in industrialised countries suffer from complaints such as allergic rhinitis, conjunctivitis or bronchial asthma. These allergies are caused by allergens present in the air (aeroallergens) which are released by sources of various origin, such as plant pollen, mites, cats or dogs. Up to 40% of these type 1 allergy sufferers in turn exhibit specific IgE reactivity with grass pollen allergens, inter alia cereal pollen allergens (Freidhoff et al., 1986, J. Allergy Clin. Immunol. 78, 1190-2001). Of the cereal pollen allergens, the allergens of rye have particular importance.

The substances which trigger type 1 allergy are proteins, glycoproteins or polypeptides. After uptake via the mucous membranes, these allergens react with the IgE molecules bonded to the surface of mast cells in sensitised individuals. If two IgE molecules are crosslinked to one another by an allergen, this results in the release of mediators (for example histamine, prostaglandins) and cytokines by the effector cell and thus in the corresponding clinical symptoms.

A distinction is made between major and minor allergens, depending on the relative frequency with which the individual allergen molecules react with the IgE antibodies of allergy sufferers.

The allergens from the pollen of various species from the family of the grasses (Poaceae) are divided into groups which are homologous amongst one another.

In particular, the molecules of major allergen group 4 have high immunological cross-reactivity with one another both with monoclonal murine antibodies and also with human IgE antibodies (Fahlbusch et al., 1993 Clin. Exp. Allergy 23:51-60; Leduc-Brodard et al., 1996, J. Allergy Clin. Immunol. 98:1065-1072; Su et al., 1996, J. Allergy Clin. Immunol. 97:210; Fahlbusch et al., 1998, Clin. Exp. Allergy 28:799-807; Gavrovic-Jankulovic et al., 2000, Invest. Allergol. Clin. Immunol. 10 (6):361-367; Stumvoll et al. 2002, Biol. Chem. 383:1383-1396; Grote et al., 2002, Biol. Chem. 383:1441-1445; Andersson and Lidholm, 2003, Int. Arch. Allergy Immunol. 130:87-107; Mari, 2003, Clin. Exp. Allergy, 33 (1):43-51).

A complete DNA sequence is hitherto not known for any of the group 4 major allergens.

From the group 4 allergen from *Dactylus glomerata*, it has hitherto only been possible for peptides to be obtained by enzymatic degradation and sequenced:

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(SEQ ID NO 13)
DIYNYMEPYVSK,

(SEQ ID NO 14)
VDPTDYFGNEQ,

(SEQ ID NO 15)
ARTAWVDSGAQLGELSY

and

GVLFNIQYVNYWFAP.
(SEQ ID NO 16, Leduc-Brodard et al., 1996, J. Allergy Clin. Immunol. 98: 1065-1072)

Peptides have also been obtained from the group 4 allergen of sub-tropical Bermuda grass (*Cynodon dactylon*) by proteolysis and sequenced:

(SEQ ID NO 17)
KTVKPLYIITP,

(SEQ ID NO 18)
KQVERDFLTSLTKDIPQLYLKs,

(SEQ ID NO 19)
TVKPLYIITPITAAMI,

(SEQ ID NO 20)
LRKYGTAADNVIDAKVVDAQGRLL,

(SEQ ID NO 21)
KWQTVAPALPDPNM,

(SEQ ID NO 22)
VTWIESVPYIPMGDK,

(SEQ ID NO 23)
GTVRDLLXRTSNIKAFGKY,

(SEQ ID NO 24)
TSNIKAFGKYKSDYVLEPIPKKS,

(SEQ ID NO 25)
YRDLDDLGVNQVVG,

(SEQ ID NO 26)
SATPPTHRSGVLFNI

and
AAAAALPTQVTRDIYAFMTPYVSKNPRQAYVNYRDLD.
(SEQ ID NO 27, Liaw et al., 2001, Biochem. Biophys. Research Communication 280: 738-743)

For *Lolium perenne*, peptide fragments having the following sequences have been described for the basic group 4 allergen: FLEPVGLIFPAGV (SEQ ID NO 28) and GLIEFPAGV (SEQ ID NO 29, Jaggi et al., 1989, Int. Arch. Allergy Appl. Immunol. 89: 342-348).

As the first sequence of a group 4 allergen, the still unpublished sequence of Phl p 4 from *Phleum pratense* (SEQ ID NO 11) has been elucidated by the inventors of the present patent application and described in International Application WO 04/000881.

Nothing is hitherto known on the sequences of the group 4 major allergens from cereals (Triceae).

The object on which the present invention was based therefore consisted in the provision of DNA sequences of group 4 major allergens from cereals, in particular the allergen Sec c 4 from rye (*Secale cereale*) (SEQ ID NO 1, 3), Hor v 4 from barley (*Hordeum vulgare*) (SEQ ID NO 5) and Tri a 4 from wheat (*Triticum aestivum*) (SEQ ID NO 7, 9) and of corresponding recombinant DNA molecules on the basis of which the allergens can be expressed as protein and made available, as such or in modified form, for pharmacologically significant

exploitation. The sequence of Phl p 4 (SEQ ID NO 11) was the starting point for the present invention.

LIST OF SEQUENCES ACCORDING TO THE INVENTION

The DNA and protein sequences of the mature allergens in accordance with SEQ ID NO 1-10 are preceded by a signal sequence. The encoding region ends with the TGA or TAG stop codons in the DNA sequences.

DNA sequence of Sec c 4. (a) Isoform Sec c 4.01 (SEQ ID NO 1), (b) isoform Sec c 4.02 (SEQ ID NO 3).

Protein sequences (SEQ ID NO 2, 4) derived from the DNA sequences in accordance with SEQ ID NO 1 and 3.

DNA sequence of Hor v 4 (SEQ ID NO 5).

Protein sequence (SEQ ID NO 6) derived from the DNA sequence in accordance with SEQ ID NO 5.

DNA sequence of Tri a 4. (a) Isoform Tri a 4.01 (SEQ ID NO 7), (b) isoform Tri a 4.02 (SEQ ID NO 9).

Protein sequences (SEQ ID NO 8, 10) derived from the DNA sequences in accordance with SEQ ID NO 7 and 9.

DNA sequence of Phl p 4 (SEQ ID NO 11), in accordance with SEQ ID NO 5 from WO 04/000881.

Protein sequence of Phl p 4 (SEQ ID NO 12), in accordance with SEQ ID NO 6 from WO 04/000881.

DESCRIPTION OF THE INVENTION

The present invention now provides for the first time DNA sequences of the cereal pollen major allergens Sec c 4, Hor v 4 and Tri a 4, in accordance with SEQ ID NO 1, 3, 5, 7, and 9.

The present invention therefore relates to DNA molecules selected from the nucleotide sequences in accordance with SEQ ID NO 1, 3, 5, 7, and 9.

The invention furthermore relates to sequences homologous to the DNA sequences according to the invention and corresponding DNA molecules of group 4 allergens from other Poaceae, such as, for example, *Lolium perenne*, *Dactylis glomerata*, *Poa pratensis*, *Cynodon dactylon* and *Holcus lanatus*, which, owing to the sequence homology that exists, hybridise with the DNA sequences according to the invention under stringent conditions, or have immunological cross-reactivity with respect to the allergens according to the invention.

The invention also encompasses fragments, new combinations of partial sequences and point mutants having a hypoallergenic action.

The invention therefore furthermore relates to corresponding partial sequences, a combination of partial sequences, or replacement, elimination or addition mutants which encode an immunomodulatory, T-cell-reactive fragment of a group 4 allergen from the Poaceae.

With knowledge of the DNA sequence of the naturally occurring allergens, it is now possible to prepare these allergens as recombinant proteins which can be used in the diagnosis and therapy of allergic diseases (Scheiner and Kraft, 1995, Allergy 50: 384-391).

A classical approach to effective therapeutic treatment of allergies is specific immunotherapy or hyposensitisation (Fiebig, 1995, Allergo J. 4 (6): 336-339, Bousquet et al., 1998, J. Allergy Clin. Immunol. 102 (4): 558-562). In this method, the patient is injected subcutaneously with natural allergen extracts in increasing doses. However, there is a risk in this method of allergic reactions or even anaphylactic shock. In order to minimise these risks, innovative preparations in the form of allergoids are employed. These are chemically modified allergen extracts which have significantly

reduced IgE reactivity, but identical T-cell reactivity compared with the untreated extract (Fiebig, 1995, Allergo J. 4 (7): 377-382).

Even more substantial therapy optimisation would be possible with allergens prepared by recombinant methods. Defined cocktails of high-purity allergens prepared by recombinant methods, optionally matched to the individual sensitisation patterns of the patients, could replace extracts from natural allergen sources since these, in addition to the various allergens, contain a relatively large number of immunogenic, but non-allergenic secondary proteins.

Realistic perspectives which may result in reliable hyposensitisation with expression products are offered by specifically mutated recombinant allergens in which IgE epitopes are specifically deleted without impairing the T-cell epitopes which are essential for therapy (Schramm et al., 1999, J. Immunol. 162: 2406-2414).

A further possibility for therapeutic influencing of the disturbed TH cell equilibrium in allergy sufferers is immunotherapeutic DNA vaccination, which involves treatment with expressible DNA which encodes the relevant allergens. Initial experimental evidence of allergen-specific influencing of the immune response has been furnished in rodents by injection of allergen-encoding DNA (Hsu et al., 1996, Nature Medicine 2 (5): 540-544).

The present invention therefore also relates to a DNA molecule described above or below as medicament and to a corresponding recombinant expression vector as medicament.

The corresponding proteins prepared by recombinant methods can be employed for therapy and for in vitro and in vivo diagnosis of pollen allergies.

For preparation of the recombinant allergen, the cloned nucleic acid is ligated into an expression vector, and this construct is expressed in a suitable host organism. After biochemical purification, this recombinant allergen is available for detection of IgE antibodies by established methods.

The present invention therefore furthermore relates to a recombinant expression vector comprising a DNA molecule described above or below, functionally linked to an expression control sequence, and a host organism transformed with said DNA molecule or said expression vector.

The invention also relates to the use of at one DNA molecule described above or at least one expression vector described above for the preparation of a medicament for the immunotherapeutic DNA vaccination of patients with allergies in the triggering of which group 4 allergens from the Poaceae, preferably Triticeae, in particular Sec c 4, Hor v 4, Tri a 4, are involved and/or for the prevention of such allergies.

As already stated, the invention can be used as an essential component in a recombinant allergen- or nucleic acid-containing preparation for specific immunotherapy. A number of possibilities arise here. On the one hand, the protein with an unchanged primary structure may be a constituent of the preparation. On the other hand, a hypoallergenic (allergoid) form can be used in accordance with the invention for therapy in order to avoid undesired side effects by specific deletion of IgE epitopes of the molecule as a whole or the production of individual fragments which encode T-cell epitopes. Finally, the nucleic acid per se, if ligated with a eukaryotic expression vector, gives a preparation which, when applied directly, modifies the allergic immune state in the therapeutic sense.

The present invention furthermore relates to the polypeptides encoded by one or more of the DNA molecules described above, preferably in their property as medicament.

These are proteins corresponding to an amino acid sequence in accordance with SEQ ID NO 2, 4, 6, 8 or 10. In

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particular, these are mature proteins (without signal sequence component), beginning with amino acid 23 (SEQ ID NO 2, 4 and 6) and with amino acid 22 (SEQ ID NO 8, 10). The invention furthermore relates to proteins which contain these amino acid sequences or parts of these sequences.

The invention accordingly also relates to a process for the preparation of such polypeptides by cultivation of a host organism and isolation of the corresponding polypeptide from the culture.

The invention likewise relates to the use of at least one polypeptide described above for the preparation of a medicament for the diagnosis and/or treatment of allergies in the triggering of which group 4 allergens from the Poaceae, preferably Triticeae, in particular Sec c 4, Hor v 4, Tri a 4, are involved and for the prevention of such allergies.

When determining the protein and DNA sequences according to the invention, the following procedure was followed:

Sec c 4 from Rye

1. Starting from the DNA sequence of Phl p 4 (SEQ ID NO 12, WO 04/000881), specific primers (Table 1) derived from the Phl p 4 sequence were generated. Five clones were obtained from rye pollen DNA by PCR with primers #87 and #83. The amplified Sec c 4 gene fragment 1 corresponding to these clones encodes a polypeptide corresponding to amino acids 68-401 of Phl p 4 (SEQ ID NO 12).

2. An EST database search was carried out with the partial Sec c 4 sequence. However, no homologous sequences were found in EST data-bases specialising in rye. Instead, individual, homologous, non-overlapping EST fragments were found in EST databases specialising in barley and wheat. Individual EST fragments extend into the 5'-UTR region and others into the 3'-UTR region (UTR=untranslated region) of the corresponding genes.

3. However, a complete group 4 gene from wheat or barley cannot be constructed from the EST sequences found in the databases since these sequences do not overlap and a homologous group 4 gene is not known. However, it was possible to assign these EST sequences with reference to the Phl p 4 sequence (SEQ ID NO 11) and the Sec c 4 fragment obtained in step 1 and these served as template for the preparation of PCR primers.

4. With the aid of primers #195 and #189 prepared in this way, three clones were obtained by PCR. Primer #195 was derived from a barley EST sequence, primer #189 is a Phl p 4-specific primer and overlaps the Phl p 4 stop codon and the codons of the 10 C-terminal Phl p 4 amino acids. The Sec c 4 gene fragment 2 amplified in this way encodes a polypeptide, beginning within the signal sequence and ending with the position corresponding to position 490 of Phl p 4. This polypeptide covers the N terminal of Sec c 4.

5a. Three further clones were obtained by PCR with primers #195 and #202. Both primers were derived from barley EST sequences. The amplified Sec c 4 gene 3 encodes the corresponding amino acids beginning within the signal sequence and ending at the C terminal of Sec c 4. The complete sequence of mature Sec c 4 is thus present in the sequence determined.

The next two steps 5b and 5c serve to double-check the result obtained in step 5a:

5b. A further clone was obtained by PCR with primers #195 and #203. Primer #195 was derived from a barley EST sequence, primer #203 from a wheat EST sequence. The amplified Sec c 4 gene encodes the corresponding amino acids beginning within the signal sequence and ending at the C terminal of Sec c 4. The complete sequence of mature Sec c 4 is therefore present in the sequence determined.

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5c. A further clone was obtained by PCR with primers #195 and #198. Also primer #198 The amplified Sec c 4 gene encodes the corresponding amino acids beginning within the signal sequence and ending at the C terminal of Sec c 4. The complete sequence of mature Sec c 4 is therefore present in the sequence determined.

Two isoforms Sec c 4.01 and 4.02 were found. The mature allergens begin with amino acid 23 of the sequences in accordance with SEQ ID NO 2, 4 and 6.

10 Hor v 4 from Barley

With the aid of the Sec c 4 sequences obtained as described above, homologous EST fragments were found in EST databases of *Hordeum vulgare*. These fragments overlap, but not to give a complete gene. With reference to the EST sequences found, however, it was possible to generate Hor v 4-specific primers, which were used for amplification of the Hor v 4 gene from genomic DNA.

In total, 15 clones were analysed.

20 4 clones were obtained by PCR with primers #195 and #198.

4 clones were obtained by PCR with primers #195 and #202.

3 clones were obtained by PCR with primers #194 and #198.

25 4 clones were obtained by PCR with primers #194 and #202.

The derived protein sequence begins within the signal sequence of Hor v 4 and extends to the C-terminal end of the 30 protein (from amino acid 23 of SEQ ID NO 6).

Tri a 4 from Wheat

With the aid of the Sec c 4 sequences obtained as described above, homologous EST fragments were found in EST databases of *Triticum aestivum*. These fragments overlap, but not to give a complete gene. With reference to the EST sequences found, however, it was possible to generate the Tri a 4-specific primers #199, #203, #204 and #206, which were used for amplification of the Tri a 4 gene from genomic DNA.

In total, 13 clones were analysed.

40 4 clones were obtained by PCR with primers #204 and #203.

4 clones were obtained by PCR with primers #204 and #199.

3 clones were obtained by PCR with primers #206 and #203.

45 4 clones were obtained by PCR with primers #206 and #199.

The derived protein sequences begin within the signal sequence of Tri a 4 and extend to the C-terminal end of the 50 protein.

Two variants Tri a 4.01 (from amino acid 22 of SEQ ID NO 8) and Tri a 4.02 (from amino acid 22 of SEQ ID NO 10) were found.

In order to prepare the recombinant allergens according to 55 the invention, the DNA sequences in accordance with SEQ ID NO 1, 3, 5, 7 and 9 were incorporated into expression vectors (for example pProEx, pSE 380). *E. coli*-optimised codons were used for the N-terminal amino acids known from the protein sequencing.

60 After transformation in *E. coli*, expression and purification of the recombinant allergens according to the invention by various separation techniques, the proteins obtained were subjected to a refolding process.

Both allergens can be employed for highly specific diagnosis of grass pollen allergies. This diagnosis can be carried out in vitro by detection of specific antibodies (IgE, IgG1-4, IgA) and reaction with IgE-loaded effector cells (for example

basophiles from blood) or in vivo by skin test reactions and provocation at the reaction organ.

The reaction of the allergens according to the invention with T-lymphocytes from grass pollen allergy sufferers can be detected by allergen-specific stimulation of the T-lymphocytes for proliferation and cytokine synthesis both with T-cells in freshly prepared blood lymphocytes and also on established nSec c 4, nHor v 4 or nTri a 4-reactive T-cell lines and clones.

The triplets encoding the cysteines were modified by site-specific mutagenesis in such a way that they encode other amino acids, preferably serine. Both variants in which individual cysteines have been replaced and those in which various combinations of 2 cysteine residues or all cysteines have been modified were prepared. The expressed proteins of these cysteine point mutants have greatly reduced or zero reactivity with IgE antibodies from allergy sufferers, but react with the T-lymphocytes from these patients.

The present invention therefore furthermore relates to a DNA molecule described above or below in which one, a plurality of or all the cysteine residues of the corresponding polypeptide have been replaced with another amino acid by site-specific mutagenesis.

The immunomodulatory activity of hypoallergenic fragments which correspond to polypeptides having T-cell epitopes and that of the hypoallergenic point mutants (for example cysteine replacements) can be detected by their reaction with T-cells from grass pollen allergy sufferers.

Such hypoallergenic fragments or point mutants of the cysteines can be employed as preparations for hyposensitisation of allergy sufferers since they react with the T-cells with equal effectiveness, but result in reduced IgE-mediated side effects owing to the reduced or entirely absent IgE reactivity.

If the nucleic acids encoding the hypoallergenic allergen variants according to the invention or the unmodified DNA molecules according to the invention are ligated with a human expression vector, these constructs can likewise be used as preparations for immunotherapy (DNA vaccination).

Finally, the present invention relates to pharmaceutical compositions comprising at least one DNA molecule described above or at least one expression vector described above and optionally further active ingredients and/or adjuvants for the immunotherapeutic DNA vaccination of patients with allergies in the triggering of which group 4 allergens from the Poaceae, preferably Triticeae, in particular Sec c 4, Hor v 4, Tri a 4, are involved and/or for the prevention of such allergies.

A further group of pharmaceutical compositions according to the invention comprises at least one polypeptide described above instead of the DNA and is suitable for the diagnosis and/or treatment of said allergies.

Pharmaceutical compositions in the sense of the present invention comprise, as active ingredients, a polypeptide according to the invention or an expression vector and/or respective pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios. The active ingredients according to the invention can be brought into a suitable

dosage form here together with at least one solid, liquid and/or semi-liquid excipient or adjuvant and optionally in combination with one or more further active ingredients.

Particularly suitable adjuvants are immunostimulatory DNA or oligonucleotides having CpG motives.

These compositions can be used as therapeutic agents or diagnostic agents in human or veterinary medicine. Suitable excipients are organic or inorganic substances which are suitable for parenteral administration and do not adversely affect the action of the active ingredient according to the invention. Suitable for parenteral use are, in particular, solutions, preferably oil-based or aqueous solutions, furthermore suspensions, emulsions or implants. The active ingredient according to the invention may also be lyophilised and the resultant lyophilisates used, for example, for the preparation of injection preparations. The compositions indicated may be sterilised and/or comprise adjuvants, such as preservatives, stabilisers and/or wetting agents, emulsifiers, salts for modifying the osmotic pressure, buffer substances and/or a plurality of further active ingredients. Furthermore, sustained-release preparations can be obtained by corresponding formulation of the active ingredient according to the invention—for example by adsorption on aluminium hydroxide.

The invention thus also serves for improving in vitro diagnosis as part of allergen component-triggering identification of the patient-specific sensitisation spectrum. The invention likewise serves for the preparation of significantly improved preparations for the specific immunotherapy of grass pollen allergies.

TABLE 1

Primers used			
	Primer number	SEQ ID NO	Sequence
a) Sec c 4			
35	#0083	30	GGCTCCGGGGCGAACCAAGTAG
	#0087	31	ACCAACGCCCTCCACATCCAGTC
40	#0189	32	GATAAGCTTCTCGAGTGATTACTACTTTT GATCAGCGGGGGATGCTC
	#0195	33	GCTCTCGATCGGCTACAATGGCG
	#0198	34	CACGCACTACAAATCTCCATGCAAG
45	#0202	35	CATGCTTGATCCTTATTCTACTAGTTGGGC
	#0203	36	TACGCACGATCCTTATTCTACTAGTTGGGC
a) Hor v 4			
50	#0194	37	GCCTTGCTCTGCCACCACGCCGCCACC
	#0195	38	GCTCTCGATCGGCTACAATGGCG
	#0198	39	CACGCACTACAAATCTCCATGCAAG
55	#0202	40	CATGCTTGATCCTTATTCTACTAGTTGGGC
c) Tri a 4			
	#0199	41	CACGCACTAAATCTCCATGCAAG
	#0203	42	TACGCACGATCCTTATTCTACTAGTTGGGC
55	#0204	43	AAGCTCTATCGGCTACAATGGCG
	#0206	44	GGTGCTCCTCTCTGCCTGTCC

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 44

<210> SEQ ID NO 1
<211> LENGTH: 1603
<212> TYPE: DNA
<213> ORGANISM: Secale cereale

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9**10**

-continued

<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1) .. (1557)

<400> SEQUENCE: 1

aac tat agg gcc ttc gcg ctg gcg ctc ctc ttc tgc gcc ttg tcc tgc	48
Asn Tyr Arg Ala Phe Ala Leu Ala Leu Leu Phe Cys Ala Leu Ser Cys	
1 5 10 15	
caa gcc gcc ggc gcc ggc tac gcg ccc gtg cct gcc aag gcg gac ttc	96
Gln Ala Ala Ala Ala Ala Tyr Ala Pro Val Pro Ala Lys Ala Asp Phe	
20 25 30	
ctc gga tgc ctc atg aag gag ata ccg gcc cgc ctc ctc tac gcc aag	144
Leu Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys	
35 40 45	
agc tcg cct gac tac ccc acc gtg ctg gcg cag acc atc agg aac tcg	192
Ser Ser Pro Asp Tyr Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser	
50 55 60	
cgg tgg tcg ccg cag aac gtg aag ccg atc tac atc atc acc ccc	240
Arg Trp Ser Ser Pro Gln Asn Val Lys Pro Ile Tyr Ile Ile Thr Pro	
65 70 75 80	
acc aac gcc tcg cac atc cag tcc gcg gtg gtg tgc ggc cgc cgg cac	288
Thr Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His	
85 90 95	
ggc atc cgc ctc cgc gtg cgg agc ggc cac gac tac gag ggc ctg	336
Gly Ile Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu	
100 105 110	
tcg tac cgg tct gag aaa ccc gag acg ttc gcc gtc gtc gac ctc aac	384
Ser Tyr Arg Ser Glu Lys Pro Glu Thr Phe Ala Val Val Asp Leu Asn	
115 120 125	
aag atg cgg gca gtg tcg gac ggc tac gcc cgc acg gcg tgg gtc	432
Lys Met Arg Ala Val Ser Val Asp Gly Tyr Ala Arg Thr Ala Trp Val	
130 135 140	
gaa tcc ggc gcg cag ctc ggc gag ctc tac tac gcg atc gcc aag aac	480
Glu Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn	
145 150 155 160	
agc ccc gtg ctc gcg ttc ccg gct ggc gtc tgc ccg tcc atc ggc gtc	528
Ser Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val	
165 170 175	
ggc ggc aac ttc gca ggc ggc ttt ggc atg ctg ctg cgc aag tac	576
Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr	
180 185 190	
ggc atc gcc gct gag aac gtc atc gac gtc aag gtg gtc gac ccc aac	624
Gly Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp Pro Asn	
195 200 205	
ggc aag ctg ctc gac aag acg tcc atg acg gcg gac cac ttc tgg gcc	672
Gly Lys Leu Leu Asp Lys Ser Ser Met Ser Ala Asp His Phe Trp Ala	
210 215 220	
gtt agg ggc ggc gga gag acg ttt ggc atc gtc gtc tgc tgg cag	720
Val Arg Gly Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln	
225 230 235 240	
gtg aag ctc ctg ccg gtg cct ccc acc gtg acc gtg ctc aag atc ccc	768
Val Lys Leu Leu Pro Val Pro Pro Thr Val Thr Val Leu Lys Ile Pro	
245 250 255	
aag acg gtg caa gaa ggc gcc ata gac ctc gtc aac aag tgg cag ctg	816
Lys Thr Val Gln Glu Gly Ala Ile Asp Leu Val Asn Lys Trp Gln Leu	
260 265 270	
gtc ggg ccg gca ctt ccc ggc gac ctc atg atc cgc atc atc ctt gcc	864
Val Gly Pro Ala Leu Pro Gly Asp Leu Met Ile Arg Ile Ile Leu Ala	
275 280 285	
ggg aac agc gcg acg ttc gag gcc atg tac ctg ggc acc tgc agt acc	912

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Gly Asn Ser Ala Thr Phe Glu Ala Met Tyr Leu Gly Thr Cys Ser Thr		
290	295	300
ctg acg ccg ctg atg agc agc aaa ttc ccc gag ctt ggc atg aac ccc		960
Leu Thr Pro Leu Met Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro		
305	310	315
320		
tcg cac tgc aac gag atg tcc tgg atc aag tcc atc ccc ttc atc cac		1008
Ser His Cys Asn Glu Met Ser Trp Ile Lys Ser Ile Pro Phe Ile His		
325	330	335
ctc ggc aag cag aac ctc gac gac ctc ctc aac cgg aac aac acc ttc		1056
Leu Gly Lys Gln Asn Leu Asp Asp Leu Leu Asn Arg Asn Asn Thr Phe		
340	345	350
aaa cca ttc gcc gaa tac aag tcg gac tac gtg tac cag ccc ttc ccc		1104
Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro		
355	360	365
aag ccc gtg tgg gag cag atc ttc ggc tgg ctt gtg aag ccc ggc gcg		1152
Lys Pro Val Trp Glu Gln Ile Phe Gly Trp Leu Val Lys Pro Gly Ala		
370	375	380
ggg atc atg atc atg gac ccc tat ggc gcc acc atc agc gct acc ccc		1200
Gly Ile Met Ile Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro		
385	390	395
400		
gaa gcg gcg acg ccg ttc cct cac cgc cag ggc gtc ctc ttc aac atc		1248
Glu Ala Ala Thr Pro Phe Pro His Arg Gln Gly Val Leu Phe Asn Ile		
405	410	415
cag tac gtc aac tac tgg ttc gct gag tca gcc ggc gcg ccg ctg		1296
Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ser Ala Gly Ala Ala Pro Leu		
420	425	430
cag tgg agc aag gac ata tac aag ttc atg gag cgc tac gtg agc aaa		1344
Gln Trp Ser Lys Asp Ile Tyr Lys Phe Met Glu Pro Tyr Val Ser Lys		
435	440	445
aat ccc agg cag gcg tat gcc aac tac agg gac atc gac ctt ggc agg		1392
Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg		
450	455	460
aat gag gtg gtg aac gac atc tcc acc tac agc agc ggc aaa gtg tgg		1440
Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val Trp		
465	470	475
480		
ggt gag aag tac ttc aag ggc aac ttccaa agg ctc gcc att acc aag		1488
Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr Lys		
485	490	495
ggc aag gtg gat cct cag gac tac ttc agg aac gag cag agc atc ccg		1536
Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro		
500	505	510
cca ctg gtc gag aag tac tga tcgaggacct tgcatggaaa tttagtgcgt		1587
Pro Leu Val Glu Lys Tyr		
515		
ggttggcggtt tcacat		1603
<210> SEQ_ID NO 2		
<211> LENGTH: 518		
<212> TYPE: PRT		
<213> ORGANISM: Secale cereale		
<400> SEQUENCE: 2		
Asn Tyr Arg Ala Phe Ala Leu Ala Leu Phe Cys Ala Leu Ser Cys		
1	5	10
15		
Gln Ala Ala Ala Ala Ala Tyr Ala Pro Val Pro Ala Lys Ala Asp Phe		
20	25	30
Leu Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys		
35	40	45
Ser Ser Pro Asp Tyr Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser		

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50	55	60
Arg Trp Ser Ser Pro Gln Asn Val Lys Pro Ile Tyr Ile Ile Thr Pro		
65	70	75
80		
Thr Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His		
85	90	95
Gly Ile Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu		
100	105	110
Ser Tyr Arg Ser Glu Lys Pro Glu Thr Phe Ala Val Val Asp Leu Asn		
115	120	125
Lys Met Arg Ala Val Ser Val Asp Gly Tyr Ala Arg Thr Ala Trp Val		
130	135	140
Glu Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn		
145	150	155
160		
Ser Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val		
165	170	175
Gly Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr		
180	185	190
Gly Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp Pro Asn		
195	200	205
Gly Lys Leu Leu Asp Lys Ser Ser Met Ser Ala Asp His Phe Trp Ala		
210	215	220
Val Arg Gly Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln		
225	230	235
240		
Val Lys Leu Leu Pro Val Pro Pro Thr Val Thr Val Leu Lys Ile Pro		
245	250	255
Lys Thr Val Gln Glu Gly Ala Ile Asp Leu Val Asn Lys Trp Gln Leu		
260	265	270
Val Gly Pro Ala Leu Pro Gly Asp Leu Met Ile Arg Ile Ile Leu Ala		
275	280	285
Gly Asn Ser Ala Thr Phe Glu Ala Met Tyr Leu Gly Thr Cys Ser Thr		
290	295	300
Leu Thr Pro Leu Met Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro		
305	310	315
320		
Ser His Cys Asn Glu Met Ser Trp Ile Lys Ser Ile Pro Phe Ile His		
325	330	335
Leu Gly Lys Gln Asn Leu Asp Asp Leu Leu Asn Arg Asn Asn Thr Phe		
340	345	350
Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro		
355	360	365
Lys Pro Val Trp Glu Gln Ile Phe Gly Trp Leu Val Lys Pro Gly Ala		
370	375	380
Gly Ile Met Ile Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro		
385	390	395
400		
Glu Ala Ala Thr Pro Phe Pro His Arg Gln Gly Val Leu Phe Asn Ile		
405	410	415
Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ser Ala Gly Ala Ala Pro Leu		
420	425	430
Gln Trp Ser Lys Asp Ile Tyr Lys Phe Met Glu Pro Tyr Val Ser Lys		
435	440	445
Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg		
450	455	460
Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val Trp		
465	470	475
480		

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Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr Lys
 485 490 495

Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro
 500 505 510

Pro Leu Val Glu Lys Tyr
 515

<210> SEQ ID NO 3
 <211> LENGTH: 1644
 <212> TYPE: DNA
 <213> ORGANISM: Secale cereale
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)...(1563)

<400> SEQUENCE: 3

aac tcg agg gcc ttt gct ctg gtg ccc ctc atc tgc gtc ttg tcc	48
Asn Ser Arg Ala Phe Ala Leu Val Pro Leu Leu Ile Cys Val Leu Ser	
1 5 10 15	
tgc cac gcc gtc tcc tac gcg gcg ccg gtg ccg gcc aag gag	96
Cys His Ala Ala Val Ser Tyr Ala Ala Pro Val Pro Ala Lys Glu	
20 25 30	
gac ttc ttc gga tgc ctg gtg aag gag ata ccg gcc cgc ctc ctc tac	144
Asp Phe Gly Cys Leu Val Lys Glu Ile Pro Ala Arg Leu Leu Tyr	
35 40 45	
gcc aag agc tcg cct gcc ttc ccc acc gtc ctg gcg cag acc atc agg	192
Ala Lys Ser Ser Pro Ala Phe Pro Thr Val Leu Ala Gln Thr Ile Arg	
50 55 60	
aac tcg cgg tgg tcg ctc ccg cag agc gtg aag ccg ctc tac atc atc	240
Asn Ser Arg Trp Ser Ser Pro Gln Ser Val Lys Pro Leu Tyr Ile Ile	
65 70 75 80	
acc ccc acc aac gcc tcc cac atc cag tcc gcg gtg tgc ggc cgc	288
Thr Pro Thr Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg	
85 90 95	
cgg cac ggc gtc cgc atc cgc gtg cgg agc ggc ggc cac gac tac gag	336
Arg His Gly Val Arg Ile Arg Val Arg Ser Gly Gly His Asp Tyr Glu	
100 105 110	
ggc ctg tcg tac cgg tcc gag cgc ccc gag ggc ttc gcc gtc gtc gac	384
Gly Leu Ser Tyr Arg Ser Glu Arg Pro Glu Ala Phe Ala Val Val Asp	
115 120 125	
ctc aac aag atg cgg gcc gtg gtg gtc gac ggc aag gct cgc acg ggc	432
Leu Asn Lys Met Arg Ala Val Val Asp Gly Lys Ala Arg Thr Ala	
130 135 140	
tgg gtg gac tcc ggt ggc cag ctc ggc gag ctc tac tac gcc atc gcc	480
Trp Val Asp Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala	
145 150 155 160	
aag aac agc ccc gtg ctc gcg ttc ccg gcc ggc gtt tgc ccg acc att	528
Lys Asn Ser Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Thr Ile	
165 170 175	
ggt gta ggc ggc aac ttc gct ggc ggc ttc ggc atg ctg ctg cgc	576
Gly Val Gly Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg	
180 185 190	
aag tac ggc atc gcc gcc gag aac gtc atc gac gtg aag gtg gtc gac	624
Lys Tyr Gly Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp	
195 200 205	
gcc aac ggc aca ctg ctc gac aag agc tcc atg agc gcg gat cac ttc	672
Ala Asn Gly Thr Leu Leu Asp Lys Ser Ser Met Ser Ala Asp His Phe	
210 215 220	
tgg gcc gtc agg ggc ggc gga gag agc ttc ggc atc gtc gtg tcg	720
Trp Ala Val Arg Gly Gly Glu Ser Phe Gly Ile Val Val Ser	

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225	230	235	240	
tgg cag gtg aag ctc ctc ccg gtg cct ccc acc gtg acc gtg ttc aag Trp Gln Val Lys Leu Leu Pro Val Pro Pro Thr Val Thr Val Phe Lys 245 250 255				768
atc ccc aag acg gtg caa gaa ggc gcc gta gag ctc atc aac aag tgg Ile Pro Lys Thr Val Gln Glu Gly Ala Val Glu Leu Ile Asn Lys Trp 260 265 270				816
cag cta gtc gcg ccg gcc ctc ccc gac gac ctg atg atc cgc atc atc Gln Leu Val Ala Pro Ala Leu Pro Asp Asp Leu Met Ile Arg Ile Ile 275 280 285				864
gct ttc ggc ggc acc gcc aag ttc gag gcc atg tac ctg ggc acc tgc Ala Phe Gly Gly Thr Ala Lys Phe Glu Ala Met Tyr Leu Gly Thr Cys 290 295 300				912
aaa gcc ctg aca ccg ctg atg agc agc aga ttc ccc gag ctc ggc atg Lys Ala Leu Thr Pro Leu Met Ser Ser Arg Phe Pro Glu Leu Gly Met 305 310 315 320				960
aac gcc tcg cac tgc aac gag atg ccc tgg atc aag tcc gtc cca ttc Asn Ala Ser His Cys Asn Glu Met Pro Trp Ile Lys Ser Val Pro Phe 325 330 335				1008
atc cac ctt ggc aag cag gcc acc ctc tcc gac ctc ctc aac cgg aac Ile His Leu Gly Lys Gln Ala Thr Leu Ser Asp Leu Leu Asn Arg Asn 340 345 350				1056
aac acc ttc aaa ccc ttc gcc gag tac aag tcg gac tac gtc tac cag Asn Thr Phe Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln 355 360 365				1104
ccc gtc ccc aag ccc gtc tgg gcg cag atc ttc gtc tgg ctc gtc aaa Pro Val Pro Lys Pro Val Trp Ala Gln Ile Phe Val Trp Leu Val Lys 370 375 380				1152
ccc ggc gcc ggg atc atg gtc atg gac ccc tac ggc gcc gcc atc agc Pro Gly Ala Gly Ile Met Val Met Asp Pro Tyr Gly Ala Ala Ile Ser 385 390 395 400				1200
gcc acc ccc gaa gcc gcc acg ccg ttc cct cac cgc aag gac gtc ctc Ala Thr Pro Glu Ala Ala Thr Pro Phe Pro His Arg Lys Asp Val Leu 405 410 415				1248
ttc aac atc cag tac gtc aac tac tgg ttc gac gag gca ggc ggc gcc Phe Asn Ile Gln Tyr Val Asn Tyr Trp Phe Asp Glu Ala Gly Gly Ala 420 425 430				1296
gcg ccg ctg cag tgg agc aag gac atg tac agg ttc atg gag ccg tac Ala Pro Leu Gln Trp Ser Lys Asp Met Tyr Arg Phe Met Glu Pro Tyr 435 440 445				1344
gtc agc aag aac ccc aga cag gcc tac gcc aac tac agg gac atc gac Val Ser Lys Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp 450 455 460				1392
ctc ggc agg aac gag gtg gtc aac gac atc tcc acc tat gcc agc ggc Leu Gly Arg Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ala Ser Gly 465 470 475 480				1440
aag gtc tgg ggc gag aag tac ttc aag ggc aac ttc caa agg ctc gcc Lys Val Trp Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala 485 490 495				1488
att acc aag ggc aag gtg gat cct cag gac tac ttc agg aac gag cag Ile Thr Lys Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln 500 505 510				1536
agc atc ccg ccg ctg cta ggg aag tag tagtactttt gcttgcatgg Ser Ile Pro Pro Leu Leu Gly Lys 515 520				1583
agatttttag tgcgttttc gcgtttcaaa tgcccaacta gtagaataag gatcgtgcgt				1643
a				1644

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<210> SEQ_ID NO 4
 <211> LENGTH: 520
 <212> TYPE: PRT
 <213> ORGANISM: Secale cereale
 <400> SEQUENCE: 4

Asn	Ser	Arg	Ala	Phe	Ala	Leu	Val	Pro	Leu	Leu	Ile	Cys	Val	Leu	Ser	
1																15
Cys	His	Ala	Ala	Val	Ser	Tyr	Ala	Ala	Ala	Pro	Val	Pro	Ala	Lys	Glu	
																20 25 30
Asp	Phe	Phe	Gly	Cys	Leu	Val	Lys	Glu	Ile	Pro	Ala	Arg	Leu	Leu	Tyr	
																35 40 45
Ala	Lys	Ser	Ser	Pro	Ala	Phe	Pro	Thr	Val	Leu	Ala	Gln	Thr	Ile	Arg	
																50 55 60
Asn	Ser	Arg	Trp	Ser	Ser	Pro	Gln	Ser	Val	Lys	Pro	Leu	Tyr	Ile	Ile	
																65 70 75 80
Thr	Pro	Thr	Asn	Ala	Ser	His	Ile	Gln	Ser	Ala	Val	Val	Cys	Gly	Arg	
																85 90 95
Arg	His	Gly	Val	Arg	Ile	Arg	Val	Arg	Ser	Gly	Gly	His	Asp	Tyr	Glu	
																100 105 110
Gly	Leu	Ser	Tyr	Arg	Ser	Glu	Arg	Pro	Glu	Ala	Phe	Ala	Val	Val	Asp	
																115 120 125
Leu	Asn	Lys	Met	Arg	Ala	Val	Val	Val	Asp	Gly	Lys	Ala	Arg	Thr	Ala	
																130 135 140
Trp	Val	Asp	Ser	Gly	Ala	Gln	Leu	Gly	Glu	Leu	Tyr	Tyr	Ala	Ile	Ala	
																145 150 155 160
Lys	Asn	Ser	Pro	Val	Leu	Ala	Phe	Pro	Ala	Gly	Val	Cys	Pro	Thr	Ile	
																165 170 175
Gly	Val	Gly	Gly	Asn	Phe	Ala	Gly	Gly	Gly	Phe	Gly	Met	Leu	Leu	Arg	
																180 185 190
Lys	Tyr	Gly	Ile	Ala	Ala	Glu	Asn	Val	Ile	Asp	Val	Lys	Val	Val	Asp	
																195 200 205
Ala	Asn	Gly	Thr	Leu	Leu	Asp	Lys	Ser	Ser	Met	Ser	Ala	Asp	His	Phe	
																210 215 220
Trp	Ala	Val	Arg	Gly	Gly	Gly	Gly	Glu	Ser	Phe	Gly	Ile	Val	Val	Ser	
																225 230 235 240
Trp	Gln	Val	Lys	Leu	Leu	Pro	Val	Pro	Pro	Thr	Val	Thr	Val	Phe	Lys	
																245 250 255
Ile	Pro	Lys	Thr	Val	Gln	Glu	Ala	Val	Glu	Leu	Ile	Asn	Lys	Trp		
																260 265 270
Gln	Leu	Val	Ala	Pro	Ala	Leu	Pro	Asp	Asp	Leu	Met	Ile	Arg	Ile	Ile	
																275 280 285
Ala	Phe	Gly	Gly	Thr	Ala	Lys	Phe	Glu	Ala	Met	Tyr	Leu	Gly	Thr	Cys	
																290 295 300
Lys	Ala	Leu	Thr	Pro	Leu	Met	Ser	Ser	Arg	Phe	Pro	Glu	Leu	Gly	Met	
																305 310 315 320
Asn	Ala	Ser	His	Cys	Asn	Glu	Met	Pro	Trp	Ile	Lys	Ser	Val	Pro	Phe	
																325 330 335
Ile	His	Leu	Gly	Lys	Gln	Ala	Thr	Leu	Ser	Asp	Leu	Leu	Asn	Arg	Asn	
																340 345 350
Asn	Thr	Phe	Lys	Pro	Phe	Ala	Glu	Tyr	Lys	Ser	Asp	Tyr	Val	Tyr	Gln	
																355 360 365
Pro	Val	Pro	Lys	Pro	Val	Trp	Ala	Gln	Ile	Phe	Val	Trp	Leu	Val	Lys	
																370 375 380

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Pro Gly Ala Gly Ile Met Val Met Asp Pro Tyr Gly Ala Ala Ile Ser
 385 390 395 400
 Ala Thr Pro Glu Ala Ala Thr Pro Phe Pro His Arg Lys Asp Val Leu
 405 410 415
 Phe Asn Ile Gln Tyr Val Asn Tyr Trp Phe Asp Glu Ala Gly Gly Ala
 420 425 430
 Ala Pro Leu Gln Trp Ser Lys Asp Met Tyr Arg Phe Met Glu Pro Tyr
 435 440 445
 Val Ser Lys Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp
 450 455 460
 Leu Gly Arg Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ala Ser Gly
 465 470 475 480
 Lys Val Trp Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala
 485 490 495
 Ile Thr Lys Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln
 500 505 510
 Ser Ile Pro Pro Leu Leu Gly Lys
 515 520

<210> SEQ ID NO 5
 <211> LENGTH: 1608
 <212> TYPE: DNA
 <213> ORGANISM: Hordeum vulgare
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1) .. (1557)
 <400> SEQUENCE: 5

agc tcg agg gcc ttc gct ctg gtg ctc ctc ctc tgc	gcc ttg tcc tgc	48	
Ser Ser Arg Ala Phe Ala Leu Val Leu Leu Cys	Ala Leu Ser Cys		
1 5	10	15	
cac cac gct gcc gtc tcc tcc gcg cag gtg ccg	gcc aag gac gac ttc	96	
His His Ala Ala Val Ser Ser Ala Gln Val Pro	Ala Lys Asp Asp Phe		
20	25	30	
ctg gga tgc ctc gtg aag gag ata ccg gcc cgc	ctc ctc ttc gcc aag	144	
Leu Gly Cys Leu Val Lys Glu Ile Pro Ala Arg	Leu Leu Phe Ala Lys		
35	40	45	
agc tcg cct gcc ttc ccc gcc gtc ctg gag cag acc	atc agg aac tcg	192	
Ser Ser Pro Ala Phe Pro Ala Val Leu Glu Gln	Thr Ile Arg Asn Ser		
50	55	60	
cgg tgg tcg tcg ccg cag aac gtg aag ccg	ctc tac atc atc acc ccc	240	
Arg Trp Ser Ser Pro Gln Asn Val Lys Pro Leu	Tyr Ile Ile Thr Pro		
65	70	75	80
acc aac acc tcc cac atc cag tct gct gtg	gtg tgc ggc cgc ccg cac	288	
Thr Asn Thr Ser His Ile Gln Ser Ala Val Val	Cys Gly Arg Arg His		
85	90	95	
ggc gtc cgc ctc cgc gtg cgg agc ggc cac	gac tac gag ggc ctg	336	
Gly Val Arg Leu Arg Val Arg Ser Gly Gly His	Asp Tyr Glu Gly Leu		
100	105	110	
tcc tac cgg tcc gag cgc ccc gag ggc ttc	gcc gtc gta gac ctc aac	384	
Ser Tyr Arg Ser Glu Arg Pro Glu Ala Phe	Ala Val Val Asp Leu Asn		
115	120	125	
aag atg cgg acc gtg ttg gtc aac gaa aag	gcc cgc acg gcg tgg	432	
Lys Met Arg Thr Val Leu Val Asn Glu Ala	Arg Thr Ala Trp Val		
130	135	140	
gac tcc ggc gcg cag ctc ggc gag ctc tac	tac gcc atc gcc aag aac	480	
Asp Ser Gly Ala Gln Leu Gly Glu Leu Tyr	Tyr Ala Ile Ala Lys Asn		
145	150	155	160
agc ccc gtg ctc gcg ttc cca gcc ggc gtt	tgc ccg tcc att ggt gta	528	

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Ser Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val		
165	170	175
ggt ggc aac ttc gct ggc ggc ttc ggc atg ctg ctg cgc aag tac	576	
Gly Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr		
180	185	190
ggc atc gcc gcc gag aac gtc atc gac gtc aag ctg gtc gac gcc aac	624	
Gly Ile Ala Ala Glu Asn Val Ile Asp Val Lys Leu Val Asp Ala Asn		
195	200	205
ggc aag ctg ctc gac aag agc tcc atg agc ccg gac cac ttc tgg gcc	672	
Gly Lys Leu Leu Asp Lys Ser Ser Met Ser Pro Asp His Phe Trp Ala		
210	215	220
gtc agg ggc ggc gga gag agc ttc ggc atc gtc gtc tcg tgg cag	720	
Val Arg Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln		
225	230	235
240		
gtg aag ctt ctc ccg gtg cct ccc acc gtg act gtg ttt cag atc ccc	768	
Val Lys Leu Leu Pro Val Pro Thr Val Thr Val Phe Gln Ile Pro		
245	250	255
aag aca gtg caa gaa ggc gcc gta gac ctc atc aac aag tgg cag ctg	816	
Lys Thr Val Gln Glu Gly Ala Val Asp Leu Ile Asn Lys Trp Gln Leu		
260	265	270
gtc gcg ccg gcc ctt ccc ggc gac atc atg atc cgc atc atc gcc atg	864	
Val Ala Pro Ala Leu Pro Gly Asp Ile Met Ile Arg Ile Ile Ala Met		
275	280	285
ggg gac aaa gcg acg ttc gag gcc atg tac ctg ggc acc tgc aaa acc	912	
Gly Asp Lys Ala Thr Phe Glu Ala Met Tyr Leu Gly Thr Cys Lys Thr		
290	295	300
ctg acg ccg ctg atg agc agc aaa ttc ccg gag ctt ggc atg aac ccc	960	
Leu Thr Pro Leu Met Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro		
305	310	315
320		
tcc cac tgc aac gag atg ccc tgg atc aag tcc atc ccc ttc atc cac	1008	
Ser His Cys Asn Glu Met Pro Trp Ile Lys Ser Ile Pro Phe Ile His		
325	330	335
ctt ggc aag cag gcc acc ctg gcc gac ctc ctc aac cgg aac aac acc	1056	
Leu Gly Lys Gln Ala Thr Leu Ala Asp Leu Leu Asn Arg Asn Asn Thr		
340	345	350
tcc aaa ccc ttc gcc gaa tac aag tcg gac tac gtc tac cag ccc gtc	1104	
Phe Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Val		
355	360	365
ccc aag ccc gtg tgg gag cag ctc ttc ggc tgg ctc acg aaa ccc ggc	1152	
Pro Lys Pro Val Trp Glu Gln Leu Phe Gly Trp Leu Thr Lys Pro Gly		
370	375	380
gct ggg atc atg gtc atg gac cca tac ggc gcc acc atc agc gcc acc	1200	
Ala Gly Ile Met Val Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr		
385	390	395
400		
ccc gaa gcg ggc acg ccg ttc cct cac cgc aag ggc gtc ctc ttc aac	1248	
Pro Glu Ala Ala Thr Pro Phe Pro His Arg Lys Gly Val Leu Phe Asn		
405	410	415
atc cag tac gtc aac tac tgg ttc gcc gag gca gcc ggc gcc ggc ccg	1296	
Ile Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ala Ala Gly Ala Ala Pro		
420	425	430
ctg cag tgg agc aag gac att tac aaa ttc atg gag ccc ttc gtg agc	1344	
Leu Gln Trp Ser Lys Asp Ile Tyr Lys Phe Met Glu Pro Phe Val Ser		
435	440	445
aag aac ccc agg cag ggc tac gcc aac tac agg gac atc gac ctc ggc	1392	
Lys Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly		
450	455	460
agg aac gag gtg gtg aac gac atc tca acc tac agc agc ggc aag gtg	1440	
Arg Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val		
465	470	475
		480

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tgg ggc gag aag tac ttc aag ggc aac ttc caa agg ctc gcc atc acc Trp Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr 485 490 495	1488
aag ggc aag gtg gat ccc cag gac tac ttc agg aac gag cag agc atc Lys Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile 500 505 510	1536
ccg ccg ctg ctg ggc aag tag tgaccgagag tcttgcattgg agatgttag Pro Pro Leu Leu Gly Lys 515	1587
tgcggtgttg gcgtttctga t	1608
<210> SEQ_ID NO 6	
<211> LENGTH: 518	
<212> TYPE: PRT	
<213> ORGANISM: Hordeum vulgare	
<400> SEQUENCE: 6	
Ser Ser Arg Ala Phe Ala Leu Val Leu Leu Cys Ala Leu Ser Cys 1 5 10 15	
His His Ala Ala Val Ser Ser Ala Gln Val Pro Ala Lys Asp Asp Phe 20 25 30	
Leu Gly Cys Leu Val Lys Glu Ile Pro Ala Arg Leu Leu Phe Ala Lys 35 40 45	
Ser Ser Pro Ala Phe Pro Ala Val Leu Glu Gln Thr Ile Arg Asn Ser 50 55 60	
Arg Trp Ser Ser Pro Gln Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro 65 70 75 80	
Thr Asn Thr Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His 85 90 95	
Gly Val Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu 100 105 110	
Ser Tyr Arg Ser Glu Arg Pro Glu Ala Phe Ala Val Val Asp Leu Asn 115 120 125	
Lys Met Arg Thr Val Leu Val Asn Glu Lys Ala Arg Thr Ala Trp Val 130 135 140	
Asp Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn 145 150 155 160	
Ser Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val 165 170 175	
Gly Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr 180 185 190	
Gly Ile Ala Ala Glu Asn Val Ile Asp Val Lys Leu Val Asp Ala Asn 195 200 205	
Gly Lys Leu Leu Asp Lys Ser Ser Met Ser Pro Asp His Phe Trp Ala 210 215 220	
Val Arg Gly Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln 225 230 235 240	
Val Lys Leu Leu Pro Val Pro Pro Thr Val Thr Val Phe Gln Ile Pro 245 250 255	
Lys Thr Val Gln Glu Gly Ala Val Asp Leu Ile Asn Lys Trp Gln Leu 260 265 270	
Val Ala Pro Ala Leu Pro Gly Asp Ile Met Ile Arg Ile Ile Ala Met 275 280 285	
Gly Asp Lys Ala Thr Phe Glu Ala Met Tyr Leu Gly Thr Cys Lys Thr 290 295 300	

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Leu Thr Pro Leu Met Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro
 305 310 315 320
 Ser His Cys Asn Glu Met Pro Trp Ile Lys Ser Ile Pro Phe Ile His
 325 330 335
 Leu Gly Lys Gln Ala Thr Leu Ala Asp Leu Leu Asn Arg Asn Asn Thr
 340 345 350
 Phe Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Val
 355 360 365
 Pro Lys Pro Val Trp Glu Gln Leu Phe Gly Trp Leu Thr Lys Pro Gly
 370 375 380
 Ala Gly Ile Met Val Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr
 385 390 395 400
 Pro Glu Ala Ala Thr Pro Phe Pro His Arg Lys Gly Val Leu Phe Asn
 405 410 415
 Ile Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ala Ala Gly Ala Ala Pro
 420 425 430
 Leu Gln Trp Ser Lys Asp Ile Tyr Lys Phe Met Glu Pro Phe Val Ser
 435 440 445
 Lys Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly
 450 455 460
 Arg Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val
 465 470 475 480
 Trp Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr
 485 490 495
 Lys Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile
 500 505 510
 Pro Pro Leu Leu Gly Lys
 515

<210> SEQ_ID NO 7
 <211> LENGTH: 1603
 <212> TYPE: DNA
 <213> ORGANISM: Triticum aestivum
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1557)

<400> SEQUENCE: 7

aac tat agg gcc ttc acg ctg gtg ctc ctc ttc tgc gcc ttg tcc tgt	48
Asn Tyr Arg Ala Phe Thr Leu Val Leu Leu Phe Cys Ala Leu Ser Cys	
1 5 10 15	
caa gcc gcc acc tac gcg ccg cct gcc aag gag gac ttc ctc	96
Gln Ala Ala Ala Thr Tyr Ala Pro Val Pro Ala Lys Glu Asp Phe Leu	
20 25 30	
ggc tgc ctc atg aag gag ata ccg gca cgc ctc tac gcc aag agc	144
Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys Ser	
35 40 45	
tgc cct gac ttc ccc acc gtc ctg gcg cag acc atc agg aac tgc cgg	192
Ser Pro Asp Phe Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser Arg	
50 55 60	
tgg ttg tcg ccg cag aac gtg aag ccg ctc tac atc acc ccc acc	240
Trp Leu Ser Pro Gln Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro Thr	
65 70 75 80	
aac gcc tcg cac atc cag tcc gcg gtg tgc gga cgc cgg cac agc	288
Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Ser	
85 90 95	
gtc cgc ctc cgc gtc cgg agc ggc ggc cac gac tac gag ggc ctg tcg	336
Val Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser	

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100	105	110														
tac	ggg	tcc	384													
Tyr	Arg	Ser	Glu	Lys	Pro	Glu	Thr	Phe	Ala	Val	Val	Asp	Leu	Asn	Lys	
115			120				125									
atg	cggttgc	atc	gac	ggc	tac	gcc	cgc	acg	gct	tgt	gtc	gaa				432
Met	Arg	Ala	Val	Leu	Ile	Asp	Gly	Tyr	Ala	Arg	Thr	Ala	Trp	Val	Glu	
130			135				140									
tcc	ggc	gag	cag	ctc	ggc	gag	ctc	tac	gcc	atc	gct	aaa	aac	agc		480
Ser	Gly	Ala	Gln	Leu	Gly	Glu	Leu	Tyr	Tyr	Ala	Ile	Ala	Lys	Asn	Ser	
145			150				155				160					
ccc	gtgttc	ccg	ttc	ccg	ggc	ggc	gtc	tgc	ccg	acc	atc	ggc	gtc	ggc		528
Pro	Val	Leu	Ala	Phe	Pro	Ala	Gly	Val	Cys	Pro	Thr	Ile	Gly	Val	Gly	
165			170				175									
ggc	aac	ttc	gca	ggc	ggc	ggc	ttt	ggc	atg	ctg	ctg	ggg	aag	tac	ggc	576
Gly	Asn	Phe	Ala	Gly	Gly	Gly	Phe	Gly	Met	Leu	Leu	Arg	Lys	Tyr	Gly	
180			185				190									
atc	gcc	gcc	gag	aac	gtc	atc	gac	gtc	aag	gtg	gtc	gac	ccc	aac	ggc	624
Ile	Ala	Ala	Glu	Asn	Val	Ile	Asp	Val	Lys	Val	Val	Asp	Pro	Asn	Gly	
195			200				205									
aag	ctt	ctc	gac	aag	agc	tcc	atg	agc	ccg	gac	cac	ttc	tgg	gcc	gtc	672
Lys	Leu	Leu	Asp	Lys	Ser	Ser	Met	Ser	Pro	Asp	His	Phe	Trp	Ala	Val	
210			215				220									
agg	ggc	ggc	ggc	gga	gag	agc	ttt	ggc	atc	gtc	gtg	tgc	tgg	caa	gtg	720
Arg	Gly	Gly	Gly	Gly	Glu	Ser	Phe	Gly	Ile	Val	Val	Ser	Trp	Gln	Val	
225			230				235			240						
aag	ctc	ctg	ccg	gtg	cct	ccc	acc	gtg	acc	gtg	ttc	aag	atc	ccc	aag	768
Lys	Leu	Leu	Pro	Val	Pro	Pro	Thr	Val	Thr	Val	Phe	Lys	Ile	Pro	Lys	
245			250				255									
aca	gtg	caa	gaa	ggc	gcc	gta	gac	ctc	gtc	aac	aag	tgg	caa	ctg	gtc	816
Thr	Val	Gln	Glu	Gly	Ala	Val	Asp	Leu	Val	Asn	Lys	Trp	Gln	Leu	Val	
260			265				270									
ggg	ccg	gcc	ctt	ccc	ggc	gac	ctc	atg	atc	cg	gtc	atc	gct	gcg	ggg	864
Gly	Pro	Ala	Leu	Pro	Gly	Asp	Leu	Met	Ile	Arg	Val	Ile	Ala	Ala	Gly	
275			280				285									
aac	acc	gct	aca	ttc	gag	ggc	atg	tac	ctg	ggc	acc	tgc	caa	acc	ctg	912
Asn	Thr	Ala	Thr	Phe	Glu	Gly	Met	Tyr	Leu	Gly	Thr	Cys	Gln	Thr	Leu	
290			295				300									
acg	ccg	ttt	atg	agc	agc	caa	ttc	ccc	gag	ctt	ggc	atg	aac	ccc	tat	960
Thr	Pro	Leu	Met	Ser	Ser	Gln	Phe	Pro	Glu	Leu	Gly	Met	Asn	Pro	Tyr	
305			310				315			320						
cac	tgc	aac	gag	atg	ccc	tgg	atc	aag	tcc	atc	ccc	ttc	atc	cac	ctc	1008
His	Cys	Asn	Glu	Met	Pro	Trp	Ile	Lys	Ser	Ile	Pro	Phe	Ile	His	Leu	
325			330				335									
ggc	aaa	gag	gcc	acc	ctg	gtc	gac	ctc	ctc	aac	cg	aa	ac	cc	ttc	1056
Gly	Lys	Glu	Ala	Ser	Leu	Val	Asp	Leu	Leu	Asn	Arg	Asn	Asn	Thr	Phe	
340			345				350									
aag	ccc	ttc	gcc	gaa	tac	aag	tcg	gac	tac	gtg	tac	cag	ccc	ttc	ccc	1104
Lys	Pro	Phe	Ala	Glu	Tyr	Lys	Ser	Asp	Tyr	Val	Tyr	Gln	Pro	Phe	Pro	
355			360				365									
aag	ccc	gtg	tgg	gag	cag	atc	ttc	ggc	tgg	ctc	acg	aag	ccc	ggt	ggg	1152
Lys	Pro	Val	Trp	Glu	Gln	Ile	Phe	Gly	Trp	Leu	Thr	Lys	Pro	Gly	Gly	
370			375				380									
ggg	atg	atg	atc	atg	gac	cca	tac	ggc	acc	atc	agc	gcc	acc	ccc		1200
Gly	Met	Met	Ile	Met	Asp	Pro	Tyr	Gly	Ala	Thr	Ser	Ile	Asp	Ala	Thr	
385			390				395			400						
gaa	cg	cg	ac	cc	tt	c	c	ac	cg	gg	gt	ct	tc	tt	ac	1248
Glu	Ala	Ala	Thr	Pro	Phe	Pro	His	Arg	Gln	Gly	Val	Leu	Phe	Asn	Ile	
405			410				415									
cag	tac	gtc	aac	tac	tgg	ttc	gcc	gag	gca	gcc	gcc	gct	ccg	ctg		1296

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Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ala Ala Ala Ala Pro Leu
420 425 430

cag tgg agc aag gac atg tac aat ttc atg gag ccg tac gtg agc aag 1344
Gln Trp Ser Lys Asp Met Tyr Asn Phe Met Glu Pro Tyr Val Ser Lys
435 440 445

aac ccc agg cag gcg tac gcc aac tac agg gac att gac ctc ggc agg 1392
Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg
450 455 460

aac gag gtg aac gac atc tca acc tat agc agc ggc aag gtt tgg 1440
Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Gly Lys Val Trp
465 470 475 480

ggc gag aag tac ttc aag ggc aac ttc caa agg ctc gct att acc aag 1488
Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr Lys
485 490 495

ggc aag gtg gat cct cag gac tac ttc agg aac gag cag agc atc ccg 1536
Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro
500 505 510

ccg ctg ctc gag aag tac tga tcgaggacct tgcatggaga ttttagtgcgt 1587
Pro Leu Leu Glu Lys Tyr
515

ggttgccgtt tcacat 1603

<210> SEQ_ID NO 8
<211> LENGTH: 518
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 8

Asn Tyr Arg Ala Phe Thr Leu Val Leu Leu Phe Cys Ala Leu Ser Cys
1 5 10 15

Gln Ala Ala Ala Thr Tyr Ala Pro Val Pro Ala Lys Glu Asp Phe Leu
20 25 30

Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys Ser
35 40 45

Ser Pro Asp Phe Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser Arg
50 55 60

Trp Leu Ser Pro Gln Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro Thr
65 70 75 80

Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Ser
85 90 95

Val Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser
100 105 110

Tyr Arg Ser Glu Lys Pro Glu Thr Phe Ala Val Val Asp Leu Asn Lys
115 120 125

Met Arg Ala Val Leu Ile Asp Gly Tyr Ala Arg Thr Ala Trp Val Glu
130 135 140

Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn Ser
145 150 155 160

Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Thr Ile Gly Val Gly
165 170 175

Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr Gly
180 185 190

Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp Pro Asn Gly
195 200 205

Lys Leu Leu Asp Lys Ser Ser Met Ser Pro Asp His Phe Trp Ala Val
210 215 220

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Arg Gly Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln Val
225 230 235 240

Lys Leu Leu Pro Val Pro Pro Thr Val Thr Val Phe Lys Ile Pro Lys
245 250 255

Thr Val Gln Glu Gly Ala Val Asp Leu Val Asn Lys Trp Gln Leu Val
260 265 270

Gly Pro Ala Leu Pro Gly Asp Leu Met Ile Arg Val Ile Ala Ala Gly
275 280 285

Asn Thr Ala Thr Phe Glu Gly Met Tyr Leu Gly Thr Cys Gln Thr Leu
290 295 300

Thr Pro Leu Met Ser Ser Gln Phe Pro Glu Leu Gly Met Asn Pro Tyr
305 310 315 320

His Cys Asn Glu Met Pro Trp Ile Lys Ser Ile Pro Phe Ile His Leu
325 330 335

Gly Lys Glu Ala Ser Leu Val Asp Leu Leu Asn Arg Asn Asn Thr Phe
340 345 350

Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro
355 360 365

Lys Pro Val Trp Glu Gln Ile Phe Gly Trp Leu Thr Lys Pro Gly Gly
370 375 380

Gly Met Met Ile Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro
385 390 395 400

Glu Ala Ala Thr Pro Phe Pro His Arg Gln Gly Val Leu Phe Asn Ile
405 410 415

Gln Tyr Val Asn Tyr Trp Phe Ala Glu Ala Ala Ala Ala Pro Leu
420 425 430

Gln Trp Ser Lys Asp Met Tyr Asn Phe Met Glu Pro Tyr Val Ser Lys
435 440 445

Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg
450 455 460

Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val Trp
465 470 475 480

Gly Glu Lys Tyr Phe Lys Gly Asn Phe Gln Arg Leu Ala Ile Thr Lys
485 490 495

Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro
500 505 510

Pro Leu Leu Glu Lys Tyr
515

<210> SEQ ID NO 9
<211> LENGTH: 1603
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1557)

<400> SEQUENCE: 9

aac tgt agg gcc ttc gcg cag gtg ctc ctc ttc gcc ttg tcc tgc	48
Asn Cys Arg Ala Phe Ala Gln Val Leu Leu Phe Phe Ala Leu Ser Cys	
1 5 10 15	
caa gcc gcc acc tac gcg ccg gtg cct gcc aag gag gac ttc ctc	96
Gln Ala Ala Ala Thr Tyr Ala Pro Val Pro Ala Lys Glu Asp Phe Leu	
20 25 30	
gga tgc ctc atg aag gag ata ccg gcc cgc ctc ctc tac gcc aag agc	144
Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys Ser	
35 40 45	

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tcg cct gac tac ccc acc gtg ctg gcg cag acc atc agg aac tcg cgg Ser Pro Asp Tyr Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser Arg 50 55 60	192
tgg tcg acg cag cag aac gtg aag ccg ctg tac atc atc acc ccc acc Trp Ser Thr Gln Gln Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro Thr 65 70 75 80	240
aac gcc tcc cac atc caa tcc gcg gtg gtg tgc ggc cgc cgcc cac ggc Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Gly 85 90 95	288
gtc cgc ctc cgc gtg cgg agc ggc cac gac tac gag ggc ctg tcg Val Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser 100 105 110	336
tac cgg tcc gag aaa ccc gag acg ttc gcc gtc gtc gac ctc aac aag Tyr Arg Ser Glu Lys Pro Glu Thr Phe Ala Val Val Asp Leu Asn Lys 115 120 125	384
atg cgg gca gtg gtt gtc gac ggc tac gcc cgc acg gcg tgg gtc gaa Met Arg Ala Val Val Val Asp Gly Tyr Ala Arg Thr Ala Trp Val Glu 130 135 140	432
tcc ggc gcg cag ctc ggc gag ctc tac tac gcc atc gcg aag aac acg Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn Ser 145 150 155 160	480
ccc gtg ctc ggc ttc ccg ggc gtc tgc ccg tcc atc ggc gtc ggc Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val Gly 165 170 175	528
ggc aac ttc gca ggc ggc ggc ttc ggc atg ctg ctg cgc aag tac ggc Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr Gly 180 185 190	576
atc gcc gcc gag aac gtc atc gac aag gtg gtc gac ccc gac ggc Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp Pro Asp Gly 195 200 205	624
aag ctg ctc gac aag acg tcc atg acg gcg gac cac ttc tgg gcc gtc Lys Leu Leu Asp Lys Ser Ser Met Ser Ala Asp His Phe Trp Ala Val 210 215 220	672
agg ggc ggc gga gag acg ttc ggc atc gtc gtc tgg cag gtg Arg Gly Gly Gly Ser Phe Gly Ile Val Val Ser Trp Gln Val 225 230 235 240	720
aag ctc atg cca gtg cct ccc acc gtc acc gtg ttt aag atc ccc aag Lys Leu Met Pro Val Pro Pro Thr Val Thr Val Phe Lys Ile Pro Lys 245 250 255	768
acg gtg caa gaa ggc gcc gta gac ctc gtc aac aag tgg cag ctg gtc Thr Val Gln Glu Gly Ala Val Asp Leu Val Asn Lys Trp Gln Leu Val 260 265 270	816
ggg ccg gca ctt ccc ggc gac ctc atg atc cgc gtc atc gct gcc ggg Gly Pro Ala Leu Pro Gly Asp Leu Met Ile Arg Val Ile Ala Ala Gly 275 280 285	864
aac acg gcg acg ttc gag gcc ttg tac ctg ggc acc tgc aaa acc ctg Asn Thr Ala Thr Phe Glu Ala Leu Tyr Leu Gly Thr Cys Lys Thr Leu 290 295 300	912
acg ccg ctg atg acg acg caa ttc ccc gag ctt ggc atg aac ccc tat Thr Pro Leu Met Ser Ser Gln Phe Pro Glu Leu Gly Met Asn Pro Tyr 305 310 315 320	960
cac tgc aac gag atg ccc tgg atc aag tcc gtc ccc ttc atc cac ctc His Cys Asn Glu Met Pro Trp Ile Lys Ser Val Pro Phe Ile His Leu 325 330 335	1008
ggc aaa cag gct ggc ctg gac gac ctc aac cgg aac aac acc ttc Gly Lys Gln Ala Gly Leu Asp Asp Leu Leu Asn Arg Asn Asn Thr Phe 340 345 350	1056
aag ccc ttc gcc gaa tac aag tgc gac tac gtg tac cag ccc ttc ccc Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro	1104

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355	360	365	
aag ccc gtg tgg gag cag atc ttc ggc tgg ctc gcg aag ccc ggc gcg Lys Pro Val Trp Glu Gln Ile Phe Gly Trp Leu Ala Lys Pro Gly Ala 370 375 380			1152
ggg atc atg atc atg gac ccc tac ggc gcc acc atc agc gcc acc ccc Gly Ile Met Ile Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro 385 390 395 400			1200
gaa gcg gcg acg ccg ttc cct cac ccg cag ggc gtc ctc ttc aac atc Glu Ala Ala Thr Pro Phe Pro His Arg Gln Gly Val Leu Phe Asn Ile 405 410 415			1248
cag tat gtc aac tac tgg ttc gcc gag cca gcc ggc gcc gcg ccg ctg Gln Tyr Val Asn Tyr Trp Phe Ala Glu Pro Ala Gly Ala Ala Pro Leu 420 425 430			1296
cag tgg agc aag gac att tac aat ttc atg gag ccg tac gtg agc aag Gln Trp Ser Lys Asp Ile Tyr Asn Phe Met Glu Pro Tyr Val Ser Lys 435 440 445			1344
aac ccc agg cag gcg tac gcc aac tac agg gac atc gac ctc ggc agg Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg 450 455 460			1392
aat gag gtg gtg aac gac atc tca acc tac agc agc ggc aag gtg tgg Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val Trp 465 470 475 480			1440
ggc gag aag tac ttc aag agc aac ttc caa agg ctc gcc att acc aag Gly Glu Lys Tyr Phe Lys Ser Asn Phe Gln Arg Leu Ala Ile Thr Lys 485 490 495			1488
ggc aag gta gat cct cag gac tac ttc agg aat gag caa agc atc ccg Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro 500 505 510			1536
ccg ctg atc gag aag tac tga tcgaggacct tgcatggaga tttagtgcgt Pro Leu Ile Glu Lys Tyr 515			1587
ggttggcggtt tcacat			1603
<210> SEQ_ID NO 10 <211> LENGTH: 518 <212> TYPE: PRT <213> ORGANISM: Triticum aestivum			
<400> SEQUENCE: 10			
Asn Cys Arg Ala Phe Ala Gln Val Leu Leu Phe Phe Ala Leu Ser Cys 1 5 10 15			
Gln Ala Ala Ala Thr Tyr Ala Pro Val Pro Ala Lys Glu Asp Phe Leu 20 25 30			
Gly Cys Leu Met Lys Glu Ile Pro Ala Arg Leu Leu Tyr Ala Lys Ser 35 40 45			
Ser Pro Asp Tyr Pro Thr Val Leu Ala Gln Thr Ile Arg Asn Ser Arg 50 55 60			
Trp Ser Thr Gln Gln Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro Thr 65 70 75 80			
Asn Ala Ser His Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Gly 85 90 95			
Val Arg Leu Arg Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser 100 105 110			
Tyr Arg Ser Glu Lys Pro Glu Thr Phe Ala Val Val Asp Leu Asn Lys 115 120 125			
Met Arg Ala Val Val Val Asp Gly Tyr Ala Arg Thr Ala Trp Val Glu 130 135 140			

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Ser Gly Ala Gln Leu Gly Glu Leu Tyr Tyr Ala Ile Ala Lys Asn Ser
 145 150 155 160
 Pro Val Leu Ala Phe Pro Ala Gly Val Cys Pro Ser Ile Gly Val Gly
 165 170 175
 Gly Asn Phe Ala Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr Gly
 180 185 190
 Ile Ala Ala Glu Asn Val Ile Asp Val Lys Val Val Asp Pro Asp Gly
 195 200 205
 Lys Leu Leu Asp Lys Ser Ser Met Ser Ala Asp His Phe Trp Ala Val
 210 215 220
 Arg Gly Gly Gly Glu Ser Phe Gly Ile Val Val Ser Trp Gln Val
 225 230 235 240
 Lys Leu Met Pro Val Pro Pro Thr Val Thr Val Phe Lys Ile Pro Lys
 245 250 255
 Thr Val Gln Glu Gly Ala Val Asp Leu Val Asn Lys Trp Gln Leu Val
 260 265 270
 Gly Pro Ala Leu Pro Gly Asp Leu Met Ile Arg Val Ile Ala Ala Gly
 275 280 285
 Asn Thr Ala Thr Phe Glu Ala Leu Tyr Leu Gly Thr Cys Lys Thr Leu
 290 295 300
 Thr Pro Leu Met Ser Ser Gln Phe Pro Glu Leu Gly Met Asn Pro Tyr
 305 310 315 320
 His Cys Asn Glu Met Pro Trp Ile Lys Ser Val Pro Phe Ile His Leu
 325 330 335
 Gly Lys Gln Ala Gly Leu Asp Asp Leu Leu Asn Arg Asn Asn Thr Phe
 340 345 350
 Lys Pro Phe Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro
 355 360 365
 Lys Pro Val Trp Glu Gln Ile Phe Gly Trp Leu Ala Lys Pro Gly Ala
 370 375 380
 Gly Ile Met Ile Met Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro
 385 390 395 400
 Glu Ala Ala Thr Pro Phe Pro His Arg Gln Gly Val Leu Phe Asn Ile
 405 410 415
 Gln Tyr Val Asn Tyr Trp Phe Ala Glu Pro Ala Gly Ala Ala Pro Leu
 420 425 430
 Gln Trp Ser Lys Asp Ile Tyr Asn Phe Met Glu Pro Tyr Val Ser Lys
 435 440 445
 Asn Pro Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg
 450 455 460
 Asn Glu Val Val Asn Asp Ile Ser Thr Tyr Ser Ser Gly Lys Val Trp
 465 470 475 480
 Gly Glu Lys Tyr Phe Lys Ser Asn Phe Gln Arg Leu Ala Ile Thr Lys
 485 490 495
 Gly Lys Val Asp Pro Gln Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro
 500 505 510
 Pro Leu Ile Glu Lys Tyr
 515

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<210> SEQ ID NO 11
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Phleum pratense
<220> FEATURE:
<221> NAME/KEY: CDS
  
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<222> LOCATION: (1)...(1503)

<400> SEQUENCE: 11

tac ttc ccg ccg gct gct aaa gaa gac ttc ctg ggt tgc ctg gtt	48
Tyr Phe Pro Pro Ala Ala Lys Glu Asp Phe Leu Gly Cys Leu Val	
1 5 10 15	
aaa gaa atc ccg ccg cgt ctg ttg tac gcg aaa tcg tcg ccg gcg tat	96
Lys Glu Ile Pro Pro Arg Leu Leu Tyr Ala Lys Ser Ser Pro Ala Tyr	
20 25 30	
ccc tca gtc ctg ggg cag acc atc cgg aac tcg agg tgg tcg tcg ccg	144
Pro Ser Val Leu Gly Gln Thr Ile Arg Asn Ser Arg Trp Ser Ser Pro	
35 40 45	
gac aac gtg aag ccg ctc tac atc acc ccc acc aac gtc tcc cac	192
Asp Asn Val Lys Pro Leu Tyr Ile Thr Pro Thr Asn Val Ser His	
50 55 60	
atc cag tcc gcc gtg gtg tgc ggc cgc cac agc gtc cgc atc cgc	240
Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Ser Val Arg Ile Arg	
65 70 75 80	
gtg cgc agc ggc ggg cac gac tac gag ggc ctc tcg tac cgg tct ttg	288
Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Arg Ser Leu	
85 90 95	
cag ccc gag acg ttc gcc gtc gac ctc aac aag atg cgg gcg gtg	336
Gln Pro Glu Thr Phe Ala Val Val Asp Leu Asn Lys Met Arg Ala Val	
100 105 110	
tgg gtg gac aag gcc cgc acg gcg tgg gtg gac tcc ggc gcg cag	384
Trp Val Asp Gly Lys Ala Arg Thr Ala Trp Val Asp Ser Gly Ala Gln	
115 120 125	
ctc ggc gag ctc tac tac gcc atc tat aag gcg agc ccc acg ctg gcg	432
Leu Gly Glu Leu Tyr Tyr Ala Ile Tyr Lys Ala Ser Pro Thr Leu Ala	
130 135 140	
tcc ccg gcc gtg tgc ccc acg atc gga gtg ggc ggc aac ttc gcg	480
Phe Pro Ala Gly Val Cys Pro Thr Ile Gly Val Gly Gly Asn Phe Ala	
145 150 155 160	
ggc ggc ggc ttc ggc atg ctg ctg cgc aag tac ggc atc gcc gcg gag	528
Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr Gly Ile Ala Ala Glu	
165 170 175	
aac gtc atc gac gtg aag ctc gtc gac gcc aac ggc aag ctg cac gac	576
Asn Val Ile Asp Val Lys Leu Val Asp Ala Asn Gly Lys Leu His Asp	
180 185 190	
aag aag tcc atg ggc gac gac cat ttc tgg gcc gtc agg ggc ggc ggg	624
Lys Lys Ser Met Gly Asp Asp His Phe Trp Ala Val Arg Gly Gly Gly	
195 200 205	
ggc gag agc ttc ggc atc gtg gtc gcg tgg cag gtg aag ctc ctg ccg	672
Gly Glu Ser Phe Gly Ile Val Val Ala Trp Gln Val Lys Leu Leu Pro	
210 215 220	
gtg ccg ccc acc gtg aca ata ttc aag atc tcc aag aca gtg agc gag	720
Val Pro Pro Thr Val Thr Ile Phe Lys Ile Ser Lys Thr Val Ser Glu	
225 230 235 240	
ggc gcc gtg gac atc atc aac aag tgg caa gtg gtc gcg ccg cag ctt	768
Gly Ala Val Asp Ile Ile Asn Lys Trp Gln Val Val Ala Pro Gln Leu	
245 250 255	
ccc gcc gac ctc atg atc cgc atc atc gcg cag ggg ccc aag gcc acg	816
Pro Ala Asp Leu Met Ile Arg Ile Ile Ala Gln Gly Pro Lys Ala Thr	
260 265 270	
ttc gag gcc atg tac ctc ggc acc tgc aaa acc ctg acg ccg ttg atg	864
Phe Glu Ala Met Tyr Leu Gly Thr Cys Lys Thr Leu Thr Pro Leu Met	
275 280 285	
agc agc aag ttc ccg gag ctc ggc atg aac ccc tcc cac tgc aac gag	912
Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro Ser His Cys Asn Glu	
290 295 300	

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atg tca tgg atc cag tcc atc ccc ttc gtc cac ctc ggc cac agg gac Met Ser Trp Ile Gln Ser Ile Pro Phe Val His Leu Gly His Arg Asp 305 310 315 320	960
gcc ctc gag gac ctc ctc aac cggaac aac tcc ttc aag ccc ttc Ala Leu Glu Asp Asp Leu Leu Asn Arg Asn Asn Ser Phe Lys Pro Phe 325 330 335	1008
gcc gaa tac aag tcc gac tac gtc tac cag ccc ttc ccc aag acc gtc Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro Lys Thr Val 340 345 350	1056
tgg gag cag atc ctc aac acc tgg ctc gtc aag ccc ggc gcc ggg atc Trp Glu Gln Ile Leu Asn Thr Trp Leu Val Lys Pro Gly Ala Gly Ile 355 360 365	1104
atg atc ttc gac ccc tac ggc gcc acc atc agc gcc acc ccg gag tcc Met Ile Phe Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro Glu Ser 370 375 380	1152
gcc acg ccc ttc cct cac cgc aag ggc gtc ctc ttc aac atc cag tac Ala Thr Pro Phe Pro His Arg Lys Gly Val Leu Phe Asn Ile Gln Tyr 385 390 395 400	1200
gtc aac tac tgg ttc gcc ccg gga gcc gcc gcg ccc ctc tcg tgg Val Asn Tyr Trp Phe Ala Pro Gly Ala Ala Ala Pro Leu Ser Trp 405 410 415	1248
agc aag gac atc tac aac tac atg gag ccc tac gtg agc aag aac ccc Ser Lys Asp Ile Tyr Asn Tyr Met Glu Pro Tyr Val Ser Lys Asn Pro 420 425 430	1296
agg cag gcg tac gca aac tac agg gac atc gac ctc ggc agg aac gag Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg Asn Glu 435 440 445	1344
gtg gtc aac gac gtc tcc acc tac gcc agc ggc aag gtc tgg ggc cag Val Val Asn Asp Val Ser Thr Tyr Ala Ser Gly Lys Val Trp Gly Gln 450 455 460	1392
aaa tac ttc aag ggc aac ttc gag agg ctc gcc att acc aag ggc aag Lys Tyr Phe Lys Gly Asn Phe Glu Arg Leu Ala Ile Thr Lys Gly Lys 465 470 475 480	1440
gtc gat cct acc gac tac ttc agg aac gag cag agc atc ccg ccg ctc Val Asp Pro Thr Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro Pro Leu 485 490 495	1488
atc aaa aag tac tga Ile Lys Lys Tyr 500	1503

<210> SEQ ID NO 12
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Phleum pratense

<400> SEQUENCE: 12

Tyr Phe Pro Pro Ala Ala Lys Glu Asp Phe Leu Gly Cys Leu Val 1 5 10 15	
Lys Glu Ile Pro Pro Arg Leu Leu Tyr Ala Lys Ser Ser Pro Ala Tyr 20 25 30	
Pro Ser Val Leu Gly Gln Thr Ile Arg Asn Ser Arg Trp Ser Ser Pro 35 40 45	
Asp Asn Val Lys Pro Leu Tyr Ile Ile Thr Pro Thr Asn Val Ser His 50 55 60	
Ile Gln Ser Ala Val Val Cys Gly Arg Arg His Ser Val Arg Ile Arg 65 70 75 80	
Val Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Arg Ser Leu 85 90 95	

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Gln Pro Glu Thr Phe Ala Val Val Asp Leu Asn Lys Met Arg Ala Val
100 105 110

Trp Val Asp Gly Lys Ala Arg Thr Ala Trp Val Asp Ser Gly Ala Gln
115 120 125

Leu Gly Glu Leu Tyr Tyr Ala Ile Tyr Lys Ala Ser Pro Thr Leu Ala
130 135 140

Phe Pro Ala Gly Val Cys Pro Thr Ile Gly Val Gly Gly Asn Phe Ala
145 150 155 160

Gly Gly Phe Gly Met Leu Leu Arg Lys Tyr Gly Ile Ala Ala Glu
165 170 175

Asn Val Ile Asp Val Lys Leu Val Asp Ala Asn Gly Lys Leu His Asp
180 185 190

Lys Lys Ser Met Gly Asp Asp His Phe Trp Ala Val Arg Gly Gly
195 200 205

Gly Glu Ser Phe Gly Ile Val Val Ala Trp Gln Val Lys Leu Leu Pro
210 215 220

Val Pro Pro Thr Val Thr Ile Phe Lys Ile Ser Lys Thr Val Ser Glu
225 230 235 240

Gly Ala Val Asp Ile Ile Asn Lys Trp Gln Val Val Ala Pro Gln Leu
245 250 255

Pro Ala Asp Leu Met Ile Arg Ile Ile Ala Gln Gly Pro Lys Ala Thr
260 265 270

Phe Glu Ala Met Tyr Leu Gly Thr Cys Lys Thr Leu Thr Pro Leu Met
275 280 285

Ser Ser Lys Phe Pro Glu Leu Gly Met Asn Pro Ser His Cys Asn Glu
290 295 300

Met Ser Trp Ile Gln Ser Ile Pro Phe Val His Leu Gly His Arg Asp
305 310 315 320

Ala Leu Glu Asp Asp Leu Leu Asn Arg Asn Asn Ser Phe Lys Pro Phe
325 330 335

Ala Glu Tyr Lys Ser Asp Tyr Val Tyr Gln Pro Phe Pro Lys Thr Val
340 345 350

Trp Glu Gln Ile Leu Asn Thr Trp Leu Val Lys Pro Gly Ala Gly Ile
355 360 365

Met Ile Phe Asp Pro Tyr Gly Ala Thr Ile Ser Ala Thr Pro Glu Ser
370 375 380

Ala Thr Pro Phe Pro His Arg Lys Gly Val Leu Phe Asn Ile Gln Tyr
385 390 395 400

Val Asn Tyr Trp Phe Ala Pro Gly Ala Ala Ala Ala Pro Leu Ser Trp
405 410 415

Ser Lys Asp Ile Tyr Asn Tyr Met Glu Pro Tyr Val Ser Lys Asn Pro
420 425 430

Arg Gln Ala Tyr Ala Asn Tyr Arg Asp Ile Asp Leu Gly Arg Asn Glu
435 440 445

Val Val Asn Asp Val Ser Thr Tyr Ala Ser Gly Lys Val Trp Gly Gln
450 455 460

Lys Tyr Phe Lys Gly Asn Phe Glu Arg Leu Ala Ile Thr Lys Gly Lys
465 470 475 480

Val Asp Pro Thr Asp Tyr Phe Arg Asn Glu Gln Ser Ile Pro Pro Leu
485 490 495

Ile Lys Lys Tyr
500

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<210> SEQ ID NO 13

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Dactylus glomerata

<400> SEQUENCE: 13

Asp	Ile	Tyr	Asn
1	5		10
Tyr Met Glu Pro Tyr Val Ser Lys			

<210> SEQ ID NO 14

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Dactylus glomerata

<400> SEQUENCE: 14

Val	Asp	Pro	Thr
1	5		10
Asp Tyr Phe Gly Asn Glu Gln			

<210> SEQ ID NO 15

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Dactylus glomerata

<400> SEQUENCE: 15

Ala	Arg	Thr	Ala
1	5		10
Trp Val Asp Ser Gly Ala Gln Leu Gly Glu Leu Ser			
			15

Tyr

<210> SEQ ID NO 16

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Dactylus glomerata

<400> SEQUENCE: 16

Gly	Val	Leu	Phe
1	5		10
Asn Ile Gln Tyr Val Asn Tyr Trp Phe Ala Pro			
			15

<210> SEQ ID NO 17

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Cynodon dactylon

<400> SEQUENCE: 17

Lys	Thr	Val	Lys
1	5		10
Pro Leu Tyr Ile Ile Thr Pro			

<210> SEQ ID NO 18

<211> LENGTH: 22

<212> TYPE: PRT

<213> ORGANISM: Cynodon dactylon

<400> SEQUENCE: 18

Lys	Gln	Val	Glu
1	5		10
Arg Asp Phe Leu Thr Ser Leu Thr Lys Asp Ile Pro			
			15

Gln	Leu	Tyr	Leu
			20
Lys Ser			

<210> SEQ ID NO 19

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Cynodon dactylon

<400> SEQUENCE: 19

Thr Val Lys Pro Leu Tyr Ile Ile Thr Pro Ile Thr Ala Ala Met Ile

<210> SEQ_ID NO 26
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Cynodon dactylon

<400> SEQUENCE: 26

Ser	Ala	Thr	Pro	Pro	Thr	His	Ser	Gly	Val	Leu	Phe	Asn	Ile
1													
													15

<210> SEQ_ID NO 27

<211> LENGTH: 36

<212> TYPE: PRT

<213> ORGANISM: Cynodon dactylon

<400> SEQUENCE: 27

Ala	Ala	Ala	Ala	Leu	Pro	Thr	Gln	Val	Thr	Arg	Asp	Ile	Tyr	Ala	Phe
1															
														15	

Met	Thr	Pro	Tyr	Val	Ser	Lys	Asn	Pro	Arg	Gln	Ala	Tyr	Val	Asn	Tyr
														20	30

Arg	Asp	Leu	Asp												
															35

<210> SEQ_ID NO 28

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Lolium perenne

<400> SEQUENCE: 28

Phe	Leu	Glu	Pro	Val	Leu	Gly	Leu	Ile	Phe	Pro	Ala	Gly	Val
1													
													10

<210> SEQ_ID NO 29

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Lolium perenne

<400> SEQUENCE: 29

Gly	Leu	Ile	Glu	Phe	Pro	Ala	Gly	Val
1								
								5

<210> SEQ_ID NO 30

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Secale cereale

<400> SEQUENCE: 30

ggctccggg gcgaaccagt ag

22

<210> SEQ_ID NO 31

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Secale cereale

<400> SEQUENCE: 31

accaacgcct cccacatcca gtc

23

<210> SEQ_ID NO 32

<211> LENGTH: 49

<212> TYPE: DNA

<213> ORGANISM: Secale cereale

<400> SEQUENCE: 32

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gataagcttc tcgagtgatt agtactttt gatcagcggc gggatgctc	49
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<210> SEQ ID NO 33
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Secale cereale
<400> SEQUENCE: 33

gctctcgatc ggctacaatg gcg	23
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<210> SEQ ID NO 34
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Secale cereale
<400> SEQUENCE: 34

cacgcactac aaatctccat gcaag	25
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<210> SEQ ID NO 35
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Secale cereale
<400> SEQUENCE: 35

catgcttgat ccttattcta cttagttggc	30
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<210> SEQ ID NO 36
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Secale cereale
<400> SEQUENCE: 36

tacgcacgat ccttattcta cttagttggc	30
----------------------------------	----

<210> SEQ ID NO 37
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare
<400> SEQUENCE: 37

gccttgtcct gccaccacgc cgccgccacc	30
----------------------------------	----

<210> SEQ ID NO 38
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare
<400> SEQUENCE: 38

gctctcgatc ggctacaatg gcg	23
---------------------------	----

<210> SEQ ID NO 39
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare
<400> SEQUENCE: 39

cacgcactac aaatctccat gcaag	25
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<210> SEQ ID NO 40
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 40

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catgcttcat ctttattcta ctatgtggc	30
<210> SEQ ID NO 41	
<211> LENGTH: 23	
<212> TYPE: DNA	
<213> ORGANISM: Triticum aestivum	
<400> SEQUENCE: 41	
cacgcactaa atctccatgc aag	23
<210> SEQ ID NO 42	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Triticum aestivum	
<400> SEQUENCE: 42	
tacgcacgat ctttattcta ctatgtggc	30
<210> SEQ ID NO 43	
<211> LENGTH: 23	
<212> TYPE: DNA	
<213> ORGANISM: Triticum aestivum	
<400> SEQUENCE: 43	
aagctctatc gcctacaatg gcg	23
<210> SEQ ID NO 44	
<211> LENGTH: 25	
<212> TYPE: DNA	
<213> ORGANISM: Triticum aestivum	
<400> SEQUENCE: 44	
ggtgcttc tcctgcgcct tgtcc	25

We claim:

1. An isolated DNA molecule comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10, wherein said nucleic acid sequence has been codon-optimized for expression of the polypeptide in a host cell that is not a cell of *Secale cereale* or *Hordeum vulgare* or *Triticum aestivum*.
2. A recombinant DNA expression vector or a cloning system comprising an isolated DNA molecule selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9, and a heterologous expression control sequence.
3. A culture comprising a host organism transformed with the recombinant DNA expression vector or cloning system of claim 2.
4. A process for the preparation of a polypeptide encoded by the isolated DNA molecule of claim 1, comprising cultivating a host organism transformed with the DNA molecule and isolating the polypeptide from the culture.
5. A method for the hyposensitization and/or immunotherapy of an allergy triggered by an allergen from Poaceae species, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 10.

- 40 species, comprising administering to a subject in need thereof an effective amount of at least one DNA molecule of claim 1.
6. A medicament comprising at least one isolated DNA molecule of claim 1 and a carrier.
7. A medicament comprising the recombinant DNA expression vector or cloning system of claim 2 and a carrier.
- 45 8. A pharmaceutical composition comprising at least one isolated DNA molecule of claim 1 and a pharmaceutically acceptable adjuvant.
9. A method for the hyposensitization and/or immunotherapy of an allergy triggered by an allergen of Poaceae species, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 8.
- 50 50 10. A pharmaceutical composition comprising at least one recombinant DNA expression vector of claim 2 and an adjuvant.
- 55 55 11. A method for the hyposensitization and/or immunotherapy of an allergy triggered by an allergen from Poaceae species, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 10.

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